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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Andrew M. Scharenberg
Serial No.:	09/869,486
Confirmation No.:	4102
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For:	CHARACTERIZATION OF THE SOC/CRAC CALCIUM CHANNEL PROTEIN FAMILY
Examiner:	Olga N. Chernyshev
Art Unit:	1646

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. 1.132

Sir:

I, Michael Y. Xie, declare that:

1. I am an employee of Synta Pharmaceuticals Corp., the exclusive licensee of the above-identified application. I have 17 years of experience in the area of ion channel drug discovery. I am currently the Associate Director of Biology, a position I have held for the past 2 years. Prior to joining Synta, I was a Senior Scientist in Lead Discovery at Millennium Pharmaceuticals, Inc. for 6 years. I have authored 16 papers on the subjects of ion channels and modulators of ion channels. My Curriculum Vitae is attached as Exhibit A.

2. I conducted a screen for modulators of the SOC3/CRAC2 channel using whole cell patch-clamp electrophysiology on HEK-293 cells. The HEK-293 cell line was used in this experiment because the cells over express the TRPM4 channel. Exhibit B shows the activation of the TRPM4 current by one of these modulators. The modulators were then tested in a murine mixed lymphocyte reaction (MLR) experiment, a recognized model for transplant rejection that is used to identify immunosuppressants. Using this model, I identified two modulators that inhibit calcium influx and are active in vivo as immunosuppressants. Exhibit C shows this immunosuppressant activity for one of these modulators. Administration of the two modulators to animals at effective therapeutic doses did not cause any observed acute toxicities or morbidity

in the animals tested, even at the highest doses tested (100 mg/kg).

3. Based on the results described herein and in the attached Exhibits, I believe that the sequences described and claimed in the instant application are useful for identifying modulators of the TRPM4 channel.

4. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued therefrom.

4/20/05
Date

Michael Y. Xie, Ph.D.

Michael Y. Xie, Ph.D.

Associate Director, Biology
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SUMMARY

- Highly motivated and independent scientific investigator and project manager; proven track record in receptor/ion channel research, assay development/HTS, and *in vivo* pharmacology for drug discovery.
- Expertise in physiology, biophysics and pharmacology of ion channels. Specialized in modern technologies of cell-based functional assays/HTS.
- Successfully characterized the FLIPR Membrane Potential Assay Kit and provided data/figures to FLIPR application note for Molecular Devices (http://www.moleculardevices.com/pages/reagents/f_mem_kit.html)
- More than 10-years industrial and postdoctoral research experiences in receptors/ion channels of central and peripheral nervous system, and recombinant receptors/ion channels expressed in mammalian cells and in *Xenopus* oocytes. Expertise in patch-clamp and two-electrode voltage-clamp recordings.
- Extensive experience in membrane and whole-cell based ligand-binding assays.
- Solid background in molecular biology and cell biology.
- Excellent communication and presentation skills.

PROFESSIONAL EXPERIENCE

Synta Pharmaceuticals Corp., Lexington, MA **06/2003 – present**

Associate Director, Biology

- Implementing ion channel drug discovery platform at Synta. Developing assays and conducting HTS for ion channel drug discovery program. Overseeing *in vitro* and *in vivo* studies of ion channel (TRP channels) drug candidates in several diseases areas (oncology, inflammation, and osteoporosis etc.). Responsible for HERG K^+ channel screening (patch-clamp) for toxicity assessment for all programs at Synta.

Millennium Pharmaceuticals, Inc., Cambridge, MA **10/1997 – 05/2003**

Senior Scientist II, HT-Biochemistry, Lead Discovery 06/2001 – 05/2003

- Implemented ion channel HTS platform and conducted one million compound screening using a fully automated FLIPR system. Responsible for hHERG K+ channel functional assay (patch-clamp) for toxicity assessment for several drug discovery programs internally. Closely interacted with pain, inflammation, cardiovascular and metabolic disease groups in target validation and drug discovery.

Senior Scientist I, Cell Biology, Lead Discovery 06/2000 - 06/2001

- Responsible for assay development for ion channels and transporters. Adapted novel screening technologies of functional cell-based assay/HTS for ion channel drug discovery. Introduced and validated novel medium throughput functional screening technologies (electrophysiology) for ion channels.
- Organized and chaired the first **Drug Discovery Technology for Ion Channels**, a satellite meeting of the Biophysical Society Annual Meeting, February 16, 2001, Cambridge, MA (This meeting has become an official annual satellite meeting of the society now).

Scientist II, Neurobiology 10/1997 - 06/2000

- Characterized novel ion channels (TRP channels and potassium channels, etc.) for Pain and CNS programs using both patch-clamp in mammalian cell lines and two-electrode voltage-clamp in *Xenopus* oocytes. Also involved in orphan GPCR de-orphaning program using both binding and functional assays.

Neurobiology Department, Harvard Medical School, Boston, MA **03/1997 – 10/1997**

Instructor

Neurobiology Department, Harvard Medical School, Boston, MA **04/1993 – 03/1997**

Postdoctoral Research Fellow

- Characterized agonist binding sites of *Torpedo* nicotinic acetylcholine receptor using both radiolabeled ligand binding assays (membrane and whole-cell preparations) as well as electrophysiological methods (two-electrode voltage-clamp for *Xenopus* oocytes and patch-clamp for mammalian cells). Employed molecular biology methods to generate mutant nicotinic acetylcholine receptors with point mutations for the studies of ligand/receptor interactions.

Northeastern University, College of Pharmacy and Allied Health Professions, Boston, MA **1994**

Lecturer of Pharmacology

- Lectured undergraduate students in Neuropharmacology.

Children's Hospital, Laboratory of Clinical Chemistry, Boston, MA **1990 – 1993**

Medical Technologist (part time)

Brigham and Women's Hospital, Laboratory of Clinical Chemistry, Boston, MA (Summers) 1988 – 1989

Medical Technician

Northeastern University, College of Pharmacy and Allied Health Professions, Boston, MA **1988 – 1993**

Teaching Assistant/Ph.D. Candidate (Department of Pharmacology)

- Characterized redox effects of naturally occurring toxins on neuronal nicotinic acetylcholine receptors in chick retinas and ciliary ganglion neurons using both ligand binding and electrophysiological methods.

Shanghai Medical College for Hospital Staff, Shanghai, P.R. China **1984 – 1987**

Instructor of Pharmacology

- Responsible for lecturing and teaching lab for senior hospital medical staff.

Shanghai Institute of Materia Medica, Laboratory of Neuropharmacology, Shanghai, P.R. China **1984**

Research Technician/Intern

HONORS

- Harvard Medical School Mahoney Fellowship Award (1993-1995).
- SmithKline Beecham Outstanding Graduate Student Award (1993) at the Twenty-second Annual Meeting of New England Pharmacologists.

EDUCATION

- Ph.D., Pharmacology, College of Pharmacy and Allied Health Professions, Northeastern University, Boston, MA
- M.D., Shanghai Medical School, Shanghai, China

INVITED ORAL PRESENTATIONS

3rd International Ion Channel Drug Targets Conference (IBC), October 18-20, 2004, San Diego, CA

Drug Discovery Technology for Ion Channels III, a satellite meeting of the Biophysical Society 47th Annual Meeting, February 28, 2003, San Antonio, Texas

Ion Channels in Drug Discovery & Development (SRI), May 20-21, 2002, Princeton, New Jersey

PharmaConference 2001: Membrane Transporters: From Identification to Drug Discovery, August 5-9, 2001, Interlaken, Switzerland

Drug Discovery Technology for Ion Channels (**Chair**), a satellite meeting of the Biophysical Society 45th Annual Meeting, February 16, 2001, Cambridge, Massachusetts

Drug Discovery Technology 2000, IBC's 5th Annual World Congress, August 14-17, 2000, Boston, MA

4th International Cell Analysis Products User Meeting (Molecular Devices). May 30 – June 2, 2000, Napa Valley, California

PUBLICATIONS

Xie, Y., Holmqvist, M.H., Hsia, A.Y. (2004) Ion channel drug discovery expands into new disease areas. *Current Drug Discovery* (April 2004): 31-33

Xu, H., Ramsey, I.S., Kotecha, S.A., Moran, M.M., Chong, J.A., Lawson, D., Ge, P., Lilly, J., Silos-Santiago, I., **Xie, Y.**, DiStefano, P.S., Curtis, R., Clapham, D.E. (2002) TRPV3 is a calcium-permeable temperature-sensitive cation channel. *Nature* 418:181-186

Baxter, D.F., Kirk, M., Garcia, A.F., Raimondi, A., Holmqvist H.M., Flint, K.K., Bojanic, D., DiStefano, P.S., Curtis, R., **Xie, Y.** (2002) A novel voltage-sensitive fluorescent dye with fast kinetics improves cell-based assays for ion channel and electrogenic transporters. *J. Biomol. Screening* 7(1): 79-85

Holmqvist, M., Cao, J., Hernandez-Pineda, R., Jacobson, M.D., Carrol, K., Sung, M.A., Jurman, M.E., Lawson, D., Ge, P., Gilbride, K., Bruke, S.L., Scannevin, R., Silos-Santiago, I., **Xie, Y.**, Covarrubias, M., Rhodes, K.J., DiStefano, P.S., An, W.F. (2002) A modulator of potassium channel inactivation domain present on an auxiliary subunit eliminates the fast inactivation of Kv4-currents. *Proc. Natl. Acad. Sci. USA* 99(2): 1035-1040

Lachnit, W., **Xie, Y.**, Curtis, R., Castle, N. (2001) Drug discovery technology for ion channels. *DDT, 6(12) (HTS suppl) S17-18*

Xie, Y. and Cohen, J.B. (2001) Contributions of *Torpedo* nicotinic acetylcholine receptor γTrp-55 and δTrp-57 to agonist and competitive antagonist function. *J. Biol. Chem.* 276(4): 2417-2426

Holmqvist, M.H., Jurman, M.E., Cao, J, Rodes, K.J., DiStefano, P.S., **Xie, Y.**, An, W.F. (2001) K-channel interacting protein dependent modulation of Kv4 current by fatty acids. *J. Neurosci.* 21(12): 4154-4161

Wang, D, Chiara, D.C., **Xie, Y.**, and Cohen, J.B. (2000) Probing the Structure of the Nicotinic Acetylcholine Receptor with 4-Benzoylbenzoylcholine, a Novel Photoaffinity Competitive Antagonist. *J. Biol. Chem.* 275(37): 28666-28674

Chiara, D.C., **Xie, Y***., and Cohen, J.B. (1999) Identification and mutational analysis of novel residues located within the binding site of *d*-tubocurarine of *Torpedo* acetylcholine receptor. *Biochemistry* 38(20): 6689-6698. **(*Joint First Author)**

Blanton, B.P., **Xie, Y.**, Dangott, L.J., and Cohen, J.B. (1999) The steroid promegestone is a non-competitive antagonist of the *Torpedo* nicotinic acetylcholine receptor which interacts with the lipid-protein interface. *Mol. Pharmacol. 55(2): 269-278.*

Xie, Y., McHugh, T., McKay, J., Jones, G.S., and Loring, R.H. (1996): Evidence that a nereistoxin metabolite, and not nereistoxin itself, reduces neuronal nicotinic receptors: Studies in the whole chick ciliary ganglion, on isolated neurons, and immunoprecipitated receptors. *J. Pharmacol. Exp. Therap. 276(1):169-177*

Xie, Y., Lane, W.V. and Loring, R.H. (1993): Nereistoxin: a naturally occurring toxin with redox effects on neuronal nicotinic receptors in chick retina. *J. Pharmacol. Exp. Therap. 264(2): 689-694*

Fisher, D.H., **Xie, Y.** and Loring, R.H. (1993): Analysis of nereistoxin using HPLC and electrochemical detection. *Analyst. Lett. 26(6): 1051-1063*

Xie, Y., Jones, G.S. and Loring, R.H. (1992): Effects of oxidizing and reducing analogs of acetylcholine on neuronal nicotinic receptors. *Molecular Pharmacol. 42: 356-363*

Rosenberg, P.A., Loring, R.H., **Xie, Y.**, Zaleska, V. and Aizenman, E. (1991): 2,4,5-Trihydroxyphenylalanine in solution forms a non-N-methyl-D-aspartate glutamatergic agonist and neurotoxin. *Proc. Natl. Acad. Sci. USA 88: 4865-4869*

Boisse, N.R., Samoriski, G.M. and **Xie, Y.** (1989): Effects of buspirone in the benzodiazepine dependent rat. *NIDA Res. Monogr. 95: 494*

ABSTRACTS

Xie, Y., Holmqvist, M.H., Yu, V., Mahiou, J., Liang, G., Shin, L., Tatsuta, N., Jiang, J., Xia, Z., Zhang, S., Chen, S., Sun, L., Hsia, A.Y., Wada, Y., and Barsoum, J. (2005) Novel, small-molecule inhibitors of CRAC channels display nanomolar potencies and primate selectivity. *Biophys. J* (submitted)

Baxter, D.F., Garcia, A.F., Raimondi, A., Jurman, M.E., Flint, K.K., Curtis, R., Kirk, M., Klaubert, D., Duzic, E., Bojanic, D., DiStefano, P.S., **Xie, Y.** (2001) A novel voltage-sensitive fluorescent dye for ion channel and transporters. *Biophys. J.* 80(2) 143a

Holmqvist, M.H., Jurman, M.E., Cao, J, **Xie, Y.**, Rodes, K.J., DiStefano, P.S., An, W.F. (2001) K-channel interacting protein dependent modulation of Kv4 current by fatty acids. *Biophys.* L. 80(2) 506a

Holmqvist, M.H., **Xie, Y.**, Jurman, M.E., Cao, J, Rodes, K.J., DiStefano, P.S., An, W.F. (2000) Kinetic modulation of Kv4-current by arachidonic acid is dependent on K-channel interacting proteins. *Soc. Neurosci. Abst. 26:1636*

An, W.F., Ling, H-P, Chen, H., **Xie, Y.**, Holmqvist, M.H., Cao, J., Jurman, M.E., Dussault, B.J., DiStefano, P.S., Betty, M., Mendoza, D., Bowlby, M.R., Rohdes, K.J. (2000) K-channel interacting protein-2 (KCHIP2) splice variants, chromosomal organization and localization. *Soc. Neurosci. Abst. 26:1635*

Blanton, B.P., Dangott, L.J., **Xie, Y.**, and Cohen, J.B. (1997) The steroid promegestone is a non-competitive antagonist of the *Torpedo* nicotinic acetylcholine receptor which interacts with the lipid-protein interface. *Biophys.J. 72(2) A152*

Wang, D., **Xie, Y**. and Cohen, J.B.: (1996): Probing the structure of the nicotinic acetylcholine receptor (nAChR) with 4-benzoylbenzoylcholine (Bz_2choline), a novel photoaffinity competitive antagonist. *Biophys. J. 70(2) A76*

Xie, Y. and Cohen, J.B. (1995): Characterization of the agonist binding sites in the *Torpedo* nAChR: contributions by the non-α subunits. *Biophys. J. 68:A406*

Xie, Y., Chiara D.C. and Cohen, J.B. (1994): Mutational analysis of novel residues identified within the binding site of *d*-tubocurarine of *Torpedo* acetylcholine receptor. *Soc. Neurosci. Abst. 20:1129*

Xie, Y., Loring, R.H. and Jones, Jr., G.S. (1993): The reduced form of nereistoxin is responsible for redox effects on neuronal nicotinic receptors (nAChRs). *Soc. Neurosci. Abst. 19:1534*

Xie, Y., Tang, L-H., Aizenman, E. and Loring, R.H. (1992): Redox effects of nereistoxin on neuronal nicotinic receptors (nAChRs) of chick ciliary ganglion. *Soc. Neurosci. Abst. 18:801*

Xie, Y. and Loring, R.H. (1991): Nereistoxin: redox effects on neuronal nicotinic receptors in chick retina. *Soc. Neurosci. Abst. 17:23*

Xie, Y., Loring, R.H. and Jones, G.S. (1990): Effects of reducing agonists on nicotinic receptors. *Soc. Neurosci. Abst. 16:205*

Loring, R.H., **Xie, Y**. and Jones, G.S. (1990): Effects of oxidizing agonists on nicotinic receptors. *Soc. Neurosci. Abst. 16:205*

Loring, R.H. and **Xie, Y**. (1989): Agmatine acts as an antagonist of nicotinic receptors. *Soc. Neurosci. Abst. 15:678*

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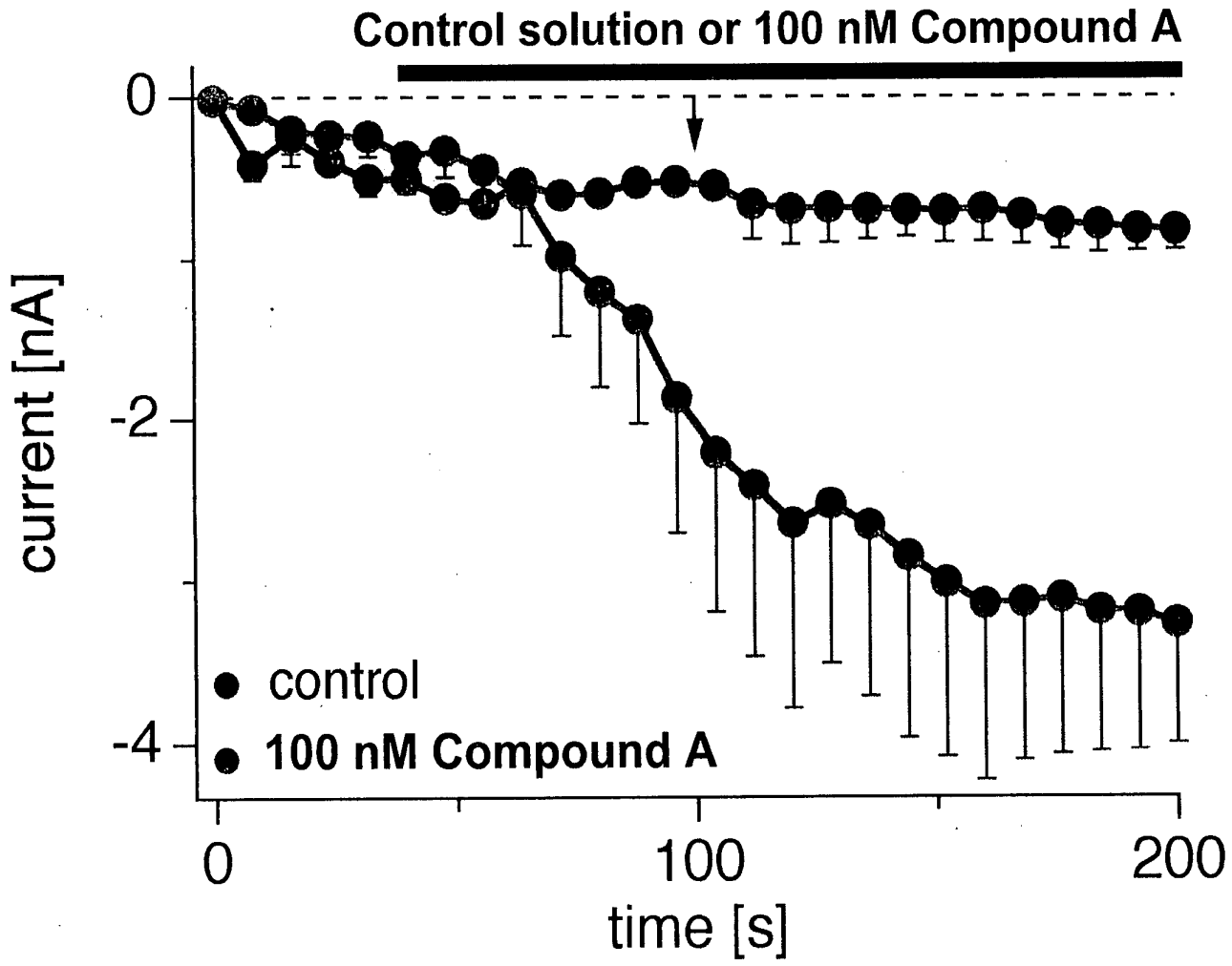
Activation of SOC3/CRAC2 Currents in HEK 293 Cells by Synta's Compound A



Synta's Compound A Was Effective in *In Vivo* MLR (Mixed Lymphocyte Reaction) in Mice